

## ORAL PRESENTATIONS

isolates (6 %) could not be further classified. For all three cultured bacteria, there was no statistically significant difference in the prevalence between conventional and 'animal-friendly' housing systems. Statistically significant differences between the two housing systems were observed for resistance to fluoroquinolones and streptomycin. Resistance to fluoroquinolones was present in 31 % of the isolates from traditional farms, but only in 20 % of the isolates from 'animal-friendly' farms. Resistance to streptomycin was common in both housing systems, but more frequent in traditional farms (85 % of the isolates) than in 'animal-friendly' farms (74 % of the isolates). When resistance to multiple antimicrobials was compared, fewer isolates from 'animal-friendly' farms were resistant to three or more of the tested antimicrobials.

**Discussion:** Our results document that for prevalence of salmonella, campylobacter and yersinia, microbiological quality of Swiss pork was on an equally high level in both conventional and 'animal-friendly' production systems. Thus, the endeavour of Swiss government and Swiss pork industry to combine 'animal-friendly' meat production with food safety was shown to have been successful. As regards antimicrobial resistance, the quality of 'animal-friendly' produced meat was even superior to conventional production. It was shown in another study on the same farms, that pigs in 'animal-friendly' farms had less injuries of the joints and injuries from tail biting than pigs in traditional farms (Cagienard et al., 2002). Fewer injuries might have resulted in a reduced use of antimicrobials. Therefore, reduced antimicrobial resistance in 'animal-friendly' production systems was probably not directly caused by differences in the housing systems, but it could be interpreted as an effect of improved pig health and therefore fewer treatments. This hypothesis will have to be investigated further.

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## O 44 *Campylobacter* species distribution in outdoor pigs

A. N. Jensen<sup>1</sup> and E. M. Nielsen<sup>2</sup>

<sup>1</sup> Danish Veterinary Institute, Bülowsvej 27, DK-1790 Copenhagen V, Denmark, tel. ++45 35300328, fax ++ 45 35300120, [anj@vetinst.dk](mailto:anj@vetinst.dk). <sup>2</sup> Statens Serum Institut, DK-2300 Copenhagen S.

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**Summary:** A colony blot hybridization method with a digoxigenin-(DIG)labelled nucleotide probe was developed to enable *Campylobacter jejuni* species-specific identification of colonies present

in minority. *C. jejuni* is the dominant cause of human cases of Campylobacteriosis. In contrast, *C. coli* is normally dominant in conventional pigs but it can be speculated that outdoor pigs host more *C. jejuni* due to a closer contact to the environment and wild-life where *C. jejuni* is pre-dominant. Since *C. jejuni* is considered more virulent to humans a potential shift towards *C. jejuni* in pigs may infer an importance to food safety. Individual pigs are often colonized with several *Campylobacter* strains. However, with conventional culturing techniques only few colonies are typically identified to the species level and thus *C. jejuni* will only rarely be isolated if present in minority. Using the hybridization method it was possible to identify *C. jejuni* colonies in 4 out of 20 faecal samples from finisher pigs.

## **Materials and Methods:** *DIG-labelled C. jejuni specific nucleotide probe:*

A primer-pair termed HIP400F and HIP1134R for amplification of *C. jejuni* (a 735 bp amplicon) was previously designed on basis of the sequence of the hippuricase (*hip*) gene, which is absent from campylobacters other than *C. jejuni*. These primers were used (1  $\mu$ M each) to prepare a *C. jejuni* specific nucleotide probe labelled with digoxigenin (DIG) using the PCR DIG Probe synthesis kit (Roche). For preparation of PCR mastermix the PCR DIG probe synthesis mix containing 0.7 mM DIG-11-dUTP was diluted 1:3 with the unlabelled dNTP stock solution. Two microlitre of a crude DNA boil-lylate of the *Campylobacter jejuni* CCUG 11284 strain was used as template DNA in the PCR. The PCR thermocycler conditions were 94 $^{\circ}$ C for 5 min; 30 cycles of 1 min at 94 $^{\circ}$ C, 1 min at 62 $^{\circ}$ C and 2 min at 72 $^{\circ}$ C and final extension for 8 min at 72 $^{\circ}$ C.

*Colony blot hybridisation:* A colony lift was made by placing a nylon membrane on an agar plate with single campylobacter colonies (<150 cfu) for 1 min. The membranes were further processed and hybridized with the DIG-labelled nucleotide probe according to colony hybridization protocol from Roche with chromogenic detection of the target-probe hybrids. The required reagents were also purchased from Roche. The pre-hybridization (1 h) and hybridization (overnight) steps were carried out at 60 $^{\circ}$ C.

*Validation of the method:* Rectal faecal samples from 10 finisher pigs (conventional) were screened for the presence of *C. coli* and *C. jejuni* with a species-specific real-time PCR (rt-PCR) (in-house test). A ten-fold dilution series was prepared by dilution of 1 g of faecal material in 9 ml of Bolton selective enrichment broth (without blood) and 0.1 ml of each dilution was spread on modified charcoal-cefoperazone-deoxycholate agar (mCCDA) plates before incubation. All incubations were performed in a microaerobic atmosphere at 41.5 $^{\circ}$ C for two days. Then 2  $\mu$ l of a crude DNA boil-lylate prepared from 1 ml of the pre-enriched Bolton broth was tested in the rt-PCR to screen for the presence of *Campylobacter jejuni* and *C. coli*, respectively. Bacterial colonies are required for hybridization and isolation of strains but the colony lift can be performed either from the direct spread on mCCDA (before pre-enrichment) or from new mCCDA plates made from the pre-enriched broth of *C. jejuni* positive sample-dilutions. However, it is essential to obtain plates with single colonies of *Campylobacter*. In this case, 0.1 ml of the enrichment broth from the *C. jejuni* positive (rt-PCR) sample were spread on mCCDA plates. After incubation, plates with less than 150 single colonies were subjected to colony hybridization as described above. Another 10 faecal samples from finisher pigs were diluted 10-fold and spread directly on mCCDA plates for colony hybridization without rt-PCR screening for *C. jejuni* beforehand.

When a target-probe hybrid was identified, presumptive *C. jejuni* colony material from the re-incubated mCCDA plate was subcultivated. The resulting isolates were biochemically tested for hydrolysis of hippurate, which distinguishes *C. jejuni* from other *Campylobacter* species. Furthermore, the isolates were identified with rt-PCR.

**Results:** The rt-PCR showed the presence of *C. coli* in all and *C. jejuni* in 2 of the 10 pre-enriched faecal samples, although one sample only yielded a weak *C. jejuni* amplification signal. The following colony hybridization of the positive sample showed that approximately half of the more than 100 cfu was target-probe hybrids. Six of the nine isolates obtained from the presumptive *C. jejuni* colonies on this mCCDA plate were tested hippurate-positive and verified as *jejuni* in the rt-PCR test. Whereas three of the *jejuni* presumptive isolates were hippurate-negative and identified as *C. coli* (rt-PCR). No target-probe hybrids were obtained from the sample with a weak *C. jejuni* amplification signal in the rt-PCR or from the negative samples.

Three of the ten samples used for direct colony hybridization showed target-probe hybrids on the colony blot membrane. These samples showed 3, 5 and 1 hybrids out of 127, 58 and 145 cfu, respectively. The isolates obtained from the identified colonies were confirmed as *C. jejuni* in rt-PCR and all but one isolate were tested hippurate-positive.

**Discussion and conclusions:** In the current study, a species-specific DIG-labelled nucleotide probe has been developed and it was possible to identify few *C. jejuni* among a majority of other *Campylobacter* spp. on mCCDA plates. However, three isolates from target-probe hybrids could not be confirmed as *C. jejuni* (*C. coli*) but this could probably be explained by difficulties in identifying the right colony or overgrowth from neighbouring colonies rather than a poor specificity of the nucleotide probe. One isolate was found hippurate-negative, however, hippurate-negative *C. jejuni* strains do exist. In this study, *C. jejuni* was isolated from 4 of the 20 examined conventional pigs, which is a relatively high incidence compared to the Danish national surveillance programme, where mainly *C. coli* is found. Hence, this method could help to isolate different *Campylobacter* species in mixed populations and not only the most predominant species, which is likely to be chosen with the conventional method where a few colonies are randomly picked.

The colony blot hybridization can be performed either on all samples without testing of the *C. jejuni* status beforehand or on those samples only, where *C. jejuni* has been found (via pre-enrichment and rt-PCR). The later reduces the number of hybridizations if only few samples contains *C. jejuni* but in addition requires preparations of DNA and PCR. Another consideration, in case of a low level of campylobacter, the pre-enrichment may be necessary to obtain any colonies for hybridization.

The colony blot hybridization method is currently used to study the *Campylobacter* species distribution in 3x16 individual pigs in an outdoor production system (experimental setup) over time (7-14 week old pigs). Despite the expected higher occurrence of *C. jejuni* in outdoor pigs due to the exposure to wild life normally hosting *C. jejuni*, the results so far only indicate very few *C. jejuni* positive pigs, although *Campylobacter* were found in all pigs.

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## O 45 Effect of an optimised pelleted diet on *Salmonella* prevalence and pig productivity

Lisbeth Jørgensen, Jaap Boes\*, Søren Krænker, Helle Kjærsgaard & Henrik Wachmann

The National Committee for Pig Production, Danish Bacon & Meat Council, Copenhagen, Denmark. Phone +45-33732679, Fax +45-33145756, E-mail: jbo@danishmeat.dk.

**Summary:** The effect of an optimised, wheat based pelleted diet containing barley, sugar beet pulp and organic acids on *Salmonella* prevalence and pig productivity was investigated in two finisher herds. The optimised diet significantly reduced *Salmonella* seroprevalence compared to standard pelleted feed and meal feed. In contrast to previous studies, meal feed did not have a significant *Salmonella* reducing effect in this study. Meal diet but not the optimised diet had a significant negative effect on pig productivity, compared to the standard pelleted diet. Our results show that the optimised diet is a suitable alternative to wheat based pelleted feed or meal feed in reducing *Salmonella* prevalence in finisher pigs.